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The Fluorescence of Indoles and Aniline Derivatives

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1. The variations in the excitation and fluorescence wavelengths and fluorescence intensities of a number of indole and aniline derivatives over a wide range of acidity and alkalinity (36N-sulphuric acid to 10N-potassium hydroxide) have been studied. 2. The changes in fluorescence with pH of the indoles and anilines had many characteristics in common, and the most fluorescent species were found to be the non-ionized or neutral forms showing fluorescence maxima at about λ 350mμ. 3. In 10N-potassium hydroxide most of the compounds examined, except those containing a tertiary nitrogen atom, showed a bathochromic shift in fluorescence wavelength attributable to an anion due to a negatively charged nitrogen, but in strong acid (3N-sulphuric acid) these compounds were non-fluorescent, except the anisidines and the 5-hydroxyindoles. 4. *p*-Anisidine but not the *o*- and *m*-isomers showed excited-state ionization in acid solution. 5. Of the hydroxyindoles only the 5-hydroxy derivatives showed a fluorescence ($\lambda_{max.}$ 520–540mμ) in acid solution. It is suggested that this fluorescence is due to a proton-transfer reaction in the excited state, and various arguments for this suggestion are given. 6. Stokes shifts for the various ionic and neutral species of the indoles and anilines have been calculated, and the large shifts found with indole and *p*-anisidine may be due to solvent–solute interaction.

Pyrrole in aqueous solution (2–100μg./ml.) is non-fluorescent (J. W. Bridges, unpublished work quoted by Williams, 1963, p. 267), but indole, which is a benzopyrrole, is highly fluorescent. In fact, several biologically important derivatives of indole are fluorescent and this property has been utilized for their quantitative estimation. However, few studies have been made of the relationship between structure and fluorescence in the indole series (e.g. Udenfriend, Bogdanski & Weissbach, 1955; White, 1959; Van Duuren, 1963; Leemann, Stich & Thomas, 1963). The present paper deals with the fluorescence of more than 20 indole derivatives in aqueous solution at various pH values and shows that the fluorescence of the indoles is similar in many respects to that of aniline and its derivatives.

EXPERIMENTAL

Materials. Aniline, b.p. 185°, *N*-methylaniline, b.p. 195°, *NN*-dimethylaniline, b.p. 193°, *p*-anisidine, b.p. 224°, *m*-anisidine, b.p. 251°, *o*-anisidine, b.p. 223° (redistilled), indole, m.p. 54·5° (from light petroleum, b.p. 40–60°), 4-benzyloxyindole, m.p. 59° (from aqueous ethanol), 5-benzyloxyindole, m.p. 103° (from water), 6-benzyloxyindole, m.p. 117° (from water), 7-benzyloxyindole, m.p. 66° (from aqueous ethanol), indol-3-ylacetic acid, m.p. 165° (from $CHCl_3$), 5-hydroxyindol-3-ylacetic acid, m.p. 164° (from $CHCl_3$), DL-tryptophan, m.p. 283° (from aqueous ethanol), 5-methoxyindole, m.p. 59° (from aqueous ethanol), indoxyl acetate, m.p. 133° (from water), and phenol, b.p. 182° (redistilled) (Koch–Light Laboratories Ltd., Colnbrook, Bucks.); 5-methylindole, m.p. 56° (from light petroleum, b.p. 60–80°) and *p*-benzyloxyaniline, m.p. 123° (from water) (Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.); physostigmine sulphate, m.p. 141° (British Drug Houses Ltd., Poole, Dorset); and *N*-methylindole, b.p. 246° (redistilled) (Fluka A.-G., Buchs SG, Switzerland), were purchased and purified as indicated. Oxindole, m.p. 127°, was synthesized from *o*-nitrophenylacetic acid (Di Carlo, 1944).

Pure samples of 5-hydroxydimethyltryptamine oxalate (bufotenine bioxalate), 6-hydroxydimethyltryptamine oxalate, 4-hydroxytryptamine (psilocin), 5-benzyloxydiethyltryptamine oxalate, 5-hydroxydiethyltryptamine, dimethyltryptamine and tryptamine hydrochloride, m.p. 256°, were given by Mr D. R. Davies (Chemical Defence Experimental Establishment, Porton Down, Wilts.). 5-Hydroxyindole was prepared by the reduction with H_2 under pressure (40lb./in.^{2}) of 5-benzyloxyindole in methanol, with 10% palladium on charcoal as catalyst. It was used immediately after the catalyst had been removed and the solvent evaporated *in vacuo*, since it darkened rapidly on exposure to air. 3,3-Dimethylindolenine picrate, m.p. 149°, was prepared from phenylhydrazine and isobutyraldehyde by the method of King, Liguori & Robinson (1934).

For the preparation of 5-methoxy-3,3-dimethylindolenine, the method of King *et al.* (1934) for the preparation of 3,3-dimethylindolenine was followed except that *p*-methoxyphenylhydrazine (12g.) and isobutyraldehyde (8·15g.)

were used. The crude product (2·6g.) was recrystallized from ethanol to give yellow needles of *5-methoxy-3,3-dimethylindolenine picrate*, m.p. 156–157° (Found: C, 50·5; H, 4·1; N, 14·0; $C_{17}H_{16}O_8N_4$ requires C, 50·5; H, 4·0; N, 13·9%).

The above compounds were checked chromatographically and were found free of impurities that might interfere with their fluorescence.

Preparation of solutions. Stock solutions were prepared by dissolving 0·1g. of the appropriate compound in 100ml. of ethanol (spectroscopic grade). For pH–fluorescence studies, stock solutions were diluted with glass-distilled water to the desired concentration (1–5μg./ml.) and examined immediately. To obtain solutions of various pH values, the aqueous solutions were titrated with 1mN- to 36N-H_2SO_4 or 1mN- to 10N-KOH. Solutions of pH 2–12 were checked with a pH-meter. Solutions of pH 0–2 and 12–14 were made up after the amounts of acid or base necessary had been calculated, assuming complete ionization. The acidity functions (H_0) of Paul & Long (1957) and of Bowden (1966) were used to express acidities below pH 0 and above pH 14 respectively. The changes in fluorescence in very strong acid or alkali were shown to be due to ionization or irreversible chemical decomposition by neutralizing the solutions and comparing the fluorescence obtained with that of fresh material in neutral solution. Fluorescence intensities were measured after N_2 had been bubbled through the solutions for 5min. to displace O_2, but in most cases the treatment did not significantly alter the intensity except with solutions of aniline, *N*-methylaniline, indole and 5-hydroxyindole, when the intensities were appreciably higher in the absence of dissolved O_2. The solutions were also examined for possible photodecomposition (cf. Bridges, Davies & Williams, 1966) but it was found that this was negligible with the compounds examined here. All intensity readings were compared with a 1cm.^{2} plastic scintillator standard of Naton 136 ($\lambda_{exc.}$ 412mμ; $\lambda_{fl.}$ 424mμ) (Thorn Electronics Ltd., New Malden, Surrey) so that they could be correlated with each other.

Spectra. Fluorescence spectra were measured in an Aminco – Bowman spectrophotofluorimeter (American Instrument Co., Silver Springs, Md., U.S.A.) and absorption spectra in a Unicam SP.500 spectrophotometer. The pK_a values were estimated from fluorescence-intensity curves as described by Bridges *et al.* (1966). The xenon lamp of the fluorimeter was calibrated by the chemical actinometer method of Hatchard & Parker (1956), excitation and fluorescence wavelengths were corrected by using a pen ray mercury lamp, and the phototube was calibrated by the method of White, Ho & Weimer (1960) and by using a front-silvered-mirror technique (Bridges, 1963). Phosphorescence spectra were determined with the phosphorimeter attachment to the Aminco–Bowman spectrophotofluorimeter. Observed and corrected values for fluorescence intensity were calculated as described by Bridges *et al.* (1966). Absolute fluorescence efficiencies (quantum efficiencies) were calculated (see Bridges & Williams, 1962) by the comparative method of Parker & Rees (1960). Quinine bisulphate in 0·1N-H_2SO_4 (1μg./ml.) was used as the fluorescence standard with a quantum efficiency of 55% (Melhuish, 1955).

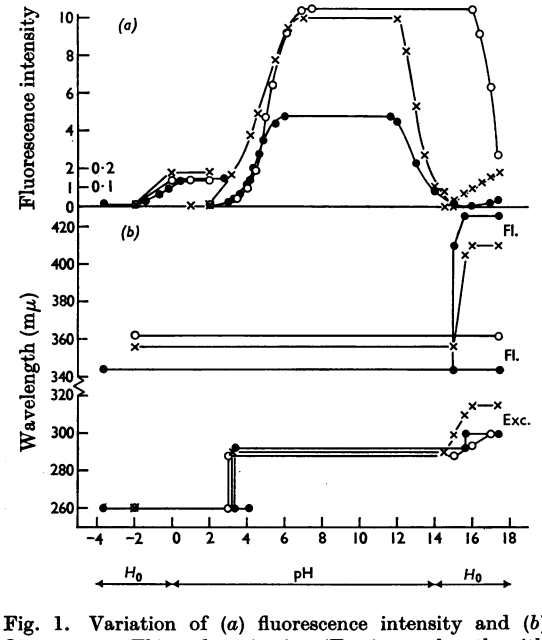


Fig. 1. Variation of (*a*) fluorescence intensity and (*b*) fluorescence (Fl.) and excitation (Exc.) wavelength with pH and H_0 of aniline (●), *N*-methylaniline (×) and *NN*-dimethylaniline (○). Intensity values are arbitrary, that of indole (1μg./ml.) at pH 7 being taken as 100. The fluorescences from H_0 −2 to pH 2 are shown at ten times their actual intensities, as indicated by the scale on the right-hand side of the intensity ordinate.

RESULTS AND DISCUSSION

Indole and aniline. Figs. 1 and 2 show that the pH–fluorescence characteristics of indole and *N*-methylindole (Fig. 2) are similar, except in

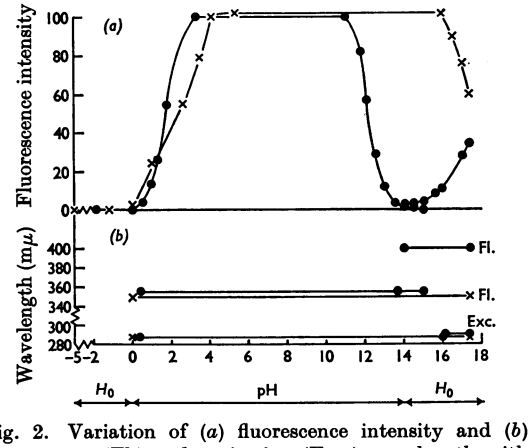
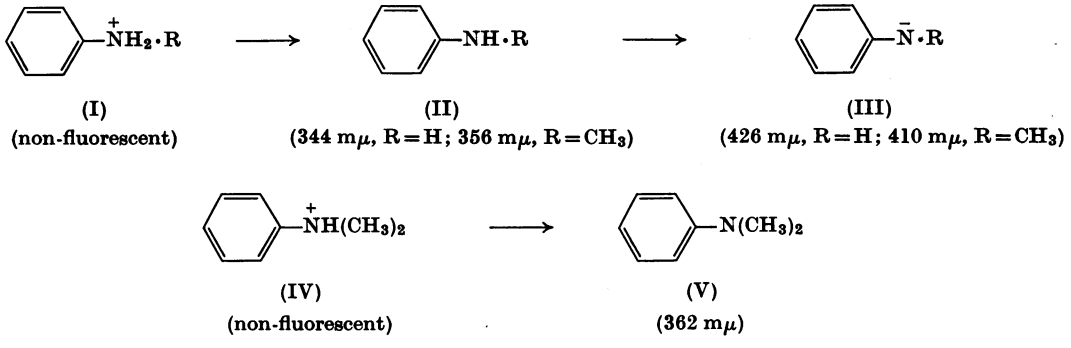
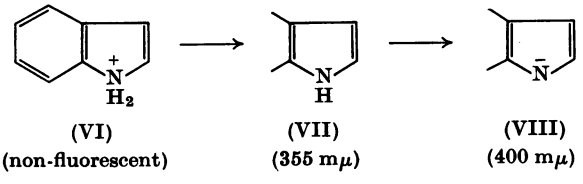


Fig. 2. Variation of (*a*) fluorescence intensity and (*b*) fluorescence (Fl.) and excitation (Exc.) wavelength with pH and H_0 of indole or 5-methylindole (●) and *N*-methylindole (×). The maximum fluorescence intensity of indole at pH 4–11 is taken as 100.





intensity, to those of *N*-methylaniline and *NN*-dimethylaniline (Fig. 1) respectively.

The main features of the fluorescence of aniline (and its *N*-methyl derivatives) from pH 0 to 14 were described earlier (Williams, 1959; Rosen & Williams, 1961), when it was shown that the anilinium cation (I, R=H) was non-fluorescent and the un-ionized form (II, R=H) was maximally fluorescent from pH 1 to 9·5. Further examination of these compounds has shown that they are also weakly fluorescent in acid solution (H_0 −1; 2N-sulphuric acid) with an intensity about 1–2% of that at neutral pH (see Fig. 1). This fluorescence is due to an excited-state ionization of the cations, for the excitation wavelength (260mμ) changes at pH 3·0–3·3 to about 290mμ, but the fluorescence wavelength (344mμ for aniline, 356mμ for *N*-methylaniline and 362mμ for *NN*-dimethylaniline) remains unchanged from H_0 −2 to 15 (3N-potassium hydroxide). The normal fluorescences of these bases begin to appear at about pH 2 and the intensity curves follow the ionization of the cations, whose pK_a values, determined from the fluorescence-intensity curves, are given in Table 1. At pH 11·6 the fluorescence of aniline begins to diminish, and it reaches zero at H_0 16 (6N-potassium hydroxide), but now a new fluorescence begins to appear at 426mμ ($\lambda_{exc.}$ 300mμ) that increases in intensity with increasing alkalinity. The fluorescence is probably due to the aniline anion (III; R=H), and this view is supported by the fact that *N*-methylaniline (II; R=CH_3) shows this alkaline fluorescence but *NN*-dimethylaniline (V) does not; its fluorescence persists undiminished in intensity to H_0 16 and then falls. The fall in the fluorescence intensity of (II) (R=H or CH_3) at about pH 12 may be due to an excited-state ionization with an inefficient emission of fluorescence. The view that an excited-state reaction is occurring is supported by the finding that in ethanolic potassium hydroxide, pH 13, the fluorescence intensity at room temperature is 25% of that in ethanol, whereas at −196° (liquid nitrogen), the intensities are the same. Excited-state reactions are diffusion-dependent and, by freezing the solution, diffusion and consequently the excited-state reaction is prevented.

In ethanol, aniline is nearly five times as fluorescent as it is in water and in mixtures of water and ethanol the intensity increases as the proportion of ethanol increases.

Indole (Fig. 2) behaves like *N*-methylaniline except that it does not appear to show fluorescence in acid solution due to excited-state ionization of the cation (VI), which is non-fluorescent. The fluorescence of the un-ionized form (VII) appears at pH 0·3 and reaches maximum intensity at pH 3·3. The pK_a of the change (VI→VII), determined from the fluorescence-intensity curve, is 1·7. The diminution in the indole fluorescence beginning at about pH 11 is, as with aniline (see above), due to an excited-state reaction, since the fall in fluorescence does not occur at −196° (liquid nitrogen) in ethanolic potassium hydroxide. The fluorescence of the indole anion (VIII) appears at pH 14 and increases in intensity to H_0 17·4 (10N-potassium hydroxide), the limit of alkalinity used. The

Table 1. *Fluorescence of aniline and indole derivatives*

The concentration used was 1 μg./ml. and in each case N_2 was bubbled through the solution before the intensity of fluorescence was measured. Fluorescence intensity was compared with that of indole at pH 7, which was taken as 100 (see the text for the meanings of observed and corrected). The calculation of absolute fluorescence efficiencies (quantum efficiencies) was based on the value of 55% for quinine bisulphate in 0·1 N-H_2SO_4 (1 μg./ml.) (see the text).

Compound	Molecular species	Wavelength (mμ)			Relative fluorescence intensity		Absolute fluorescence efficiency (%)	pH range of maximum fluorescence	pK_a	
		Of max. absorption	Of max. excitation	Of max. fluorescence	Observed	Corrected			From fluorescence intensity	Literature values
Aniline	Cation	254	—	—	—	—	—	—	4·5	4·58*
	Cation (excited)	254	260	344	0·15	0·38	0·3	pH 1·0–8·4	−0·5	
	Neutral form	281	294	344	4·8	4·0	2·4	pH 6·0–11·7	>16	
	Anion	296	300	426	0·4	0·29	0·15	at H_0 17·4	—	
N-Methylaniline	Cation	254	—	—	—	—	—	—	4·6	4·85*
	Cation (excited)	254	260	356	0·18	0·46	0·35	pH 0–2		
	Neutral form	288	290	356	10·0	9·4	5·1	pH 7–12	>14·5	
	Anion	310	315	410	1·8	0·98	0·5	at H_0 17·4	—	
NN-Dimethylaniline	Cation	254	—	—	—	—	—	—	5·1	5·06*
	Cation (excited)	254	260	362	0·14	0·35	0·2	pH 0–2	—	
	Neutral form	291	288	362	10·5	10·3	0·6	pH 6·6–H_0 16		
Indole or 5-methylindole	Cation	269	—	—	—	—	—	—	1·7	−2·4?†
	Neutral form	270	287	355	100·0	100·0	46·0	pH 3·3–11·0	>14	
	Anion	280	290	400	35·0	32·0	14·5	at H_0 17·4	—	
N-Methylindole	Cation	280	—	—	—	—	—	—	2·5	
	Neutral form	282	287	350	102·0	102·0	47·0	pH 4·3–H_0 16	—	
Tryptamine or dimethyltryptamine	Dication	280	—	—	—	—	—	—	1·6	
	Cation	280	290	360	75·5	71·0	35·0	pH 4–9	9·65	10·2?†
	Neutral form	282	291	362	87·5	78·5	37·3	at pH 10·3	>15	
	Anion	291	291	408	12·0	10·8	5·1	at H_0 17	—	
Tryptophan	Dication	280	—	—	—	—	—	—	0·5	
	Cation	280	286	345	~5·0	~16·0	9·1	pH 1	2·45	2·38‡
	Zwitterion	278	287	352	25·0	25·0	14·9	pH 4·1–8·6	9·6	9·62‡
	Anion	278	289	359	66·5	63·5	28·9	pH 10·7–11·1	>15	
	Dianion	280	292	411	14·0	12·3	7·2	at H_0 17		
Indol-3-ylacetic acid	Cation	279	—	—	—	—	—	—	1·7	
	Neutral form	279	292	356	~75–80	~66–71	30·7	~pH 3·5–4·0	4·4	3·7§
	Anion	280	292	362	125	110	56·5	pH 5·9–10·6	>14	
	Dianion	280	292	415	25·0	22·0	10·1	at H_0 17·5		
5-Hydroxyindol-3-ylacetic acid	Cation	~298	—	—	—	—	—	—	0·4	
	Cation (excited)	~298	300	545	0·33	0·23	0·1	at H_0 −1·0		
	Neutral form	~298	306	342	20·0	12·5	5·6	~pH 2–3	4·6	
	Anion	~298	306	350	63·0	34·4	15·5	pH 5·9–9·9	11·2	
	Dianion	~298	306	350	0	0	—	pH 12·4–14·0	>14	
	Trianion	315	328	400–407	9·0	4·0	1·8	at H_0 17·4		

* Albert & Serjeant (1962); † Perrin (1965); ‡ Meister (1957); § Milne, Crawford, Girao & Loughbridge (1960).

fluorescence wavelengths of (VII) (355 mμ) and (VIII) (400 mμ) are similar to those of (II) (R = CH_3; 356 mμ) and (III) (R = CH_3; 410 mμ), as might be expected. The fluorescence of (VII) reaches zero at H_0 15 and between pH 14 and H_0 15 indole fluoresces at 355 and 400 mμ. 5-Methylindole (IX) (Fig. 2) fluoresces exactly like indole, but *N*-methylindole (XI) shows but one fluorescence at 350 mμ ($\lambda_{exc.}$ 287 mμ), which is maximal in intensity from pH 4·5 to H_0 16; its behaviour recalls that of *NN*-dimethylaniline. The pK_a of the change (X→XI), from the fluorescence-intensity curve (Fig. 2), is 2·5.

The observed fluorescence intensities of the un-ionized forms of indole and *N*-methylindole are about ten times those of the methylanilines, which are themselves twice as intense as that of aniline.

Tryptamine and tryptophan. The pH–fluorescence curves (Fig. 3) of tryptamine (XIII) (or dimethyltryptamine, XVI) and tryptophan (XVII) show evidence of four molecular species of tryptamine and five for tryptophan.

The dication (XII) is non-fluorescent. At pH 0, the monocation (XIII) appears and fluoresces maximally from pH 4 to 9. At pH 9, the fluorescence of the un-ionized form (XIV) appears and reaches maximum intensity at pH 10·4 and then diminishes to zero just below H_0 16. The fluorescence of the anion (XV) occurs at H_0 16–17. From the

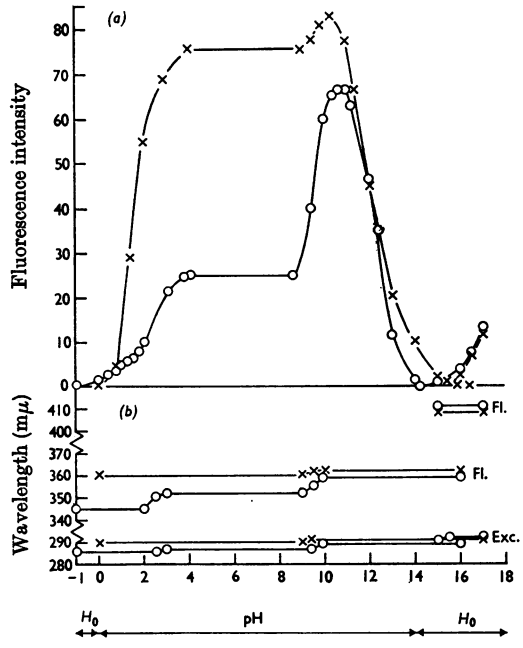
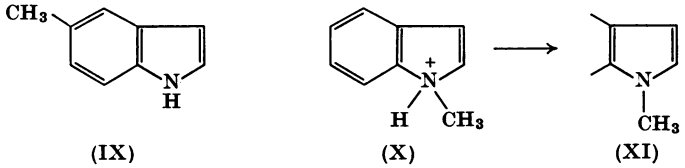
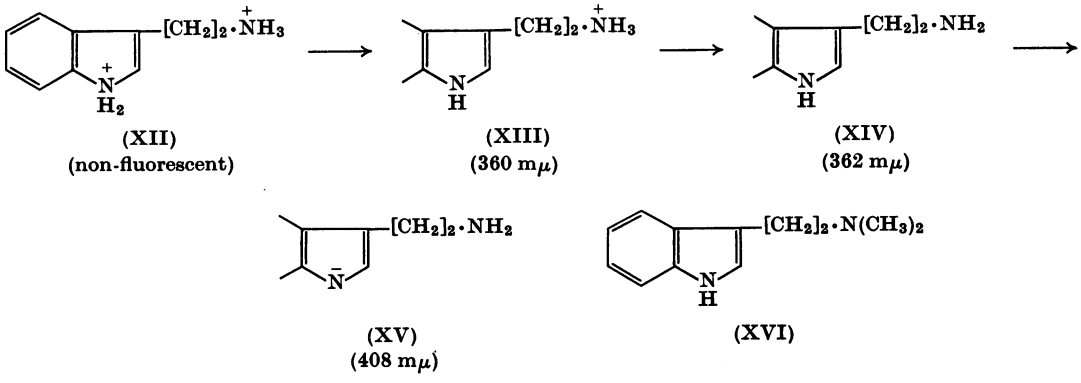
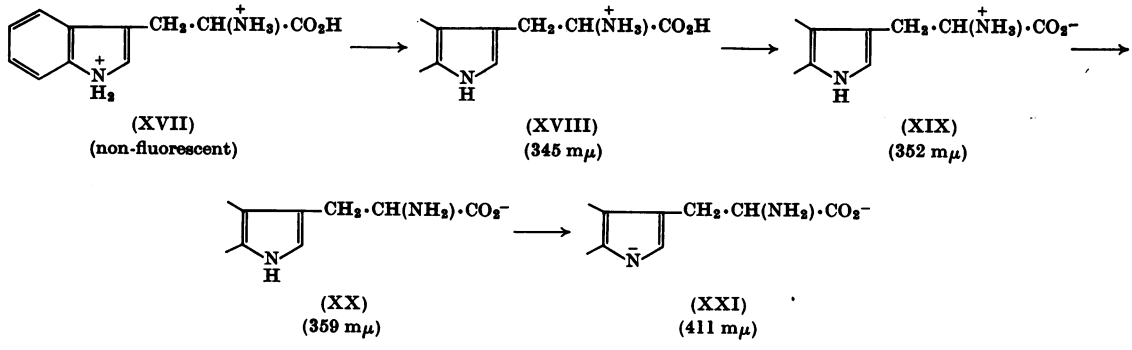


Fig. 3. Variation of (*a*) fluorescence intensity and (*b*) fluorescence (Fl.) and excitation (Exc.) wavelength with pH and H_0 of tryptophan (○) and tryptamine or *NN*-dimethyltryptamine (×). Intensity values are arbitrary, that of indole (1 μg./ml.) at pH 7 being taken as 100.







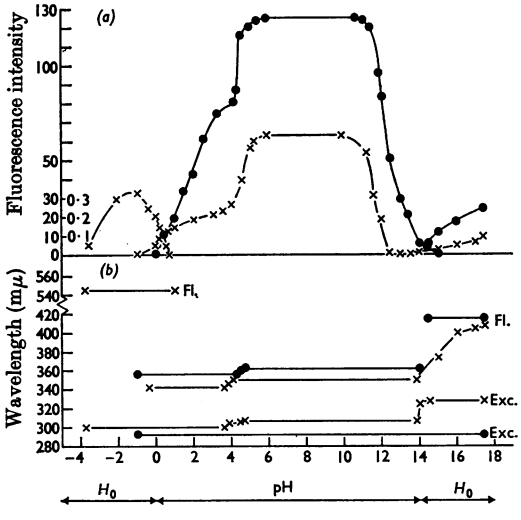


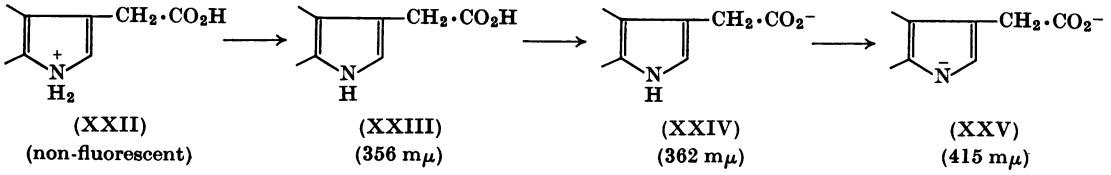
Fig. 4. Variation of (*a*) fluorescence intensity and (*b*) fluorescence (Fl.) and excitation (Exc.) wavelength with pH and H_0 of indol-3-ylacetic acid (●) and 5-hydroxyindol-3-ylacetic acid (×). Intensity values are arbitrary; that of indole at pH 7 is taken as 100. The fluorescence intensity of 5-hydroxyindol-3-ylacetic acid at 545 mμ from H_0 −3·75 to pH 1 is shown at 100 times the actual values, as indicated by the scale on the right side of the intensity ordinate.

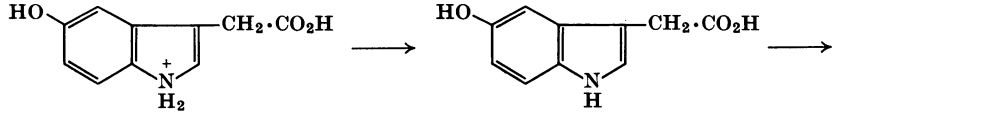
fluorescence-intensity curve the pK_a of each of these changes can be estimated (see Table 1). The tryptophan ionization is shown in (XVII) to (XXI). The change (XVII→XVIII) occurs from H_0 −1 to pH 1 and the change (XVIII→XIX) from pH 1 to 4. The zwitterion (XIX) fluoresces maximally from pH 4·1 to 8·6 with an intensity that is 25% of that of indole. The anion (XX) is much more intensely fluorescent, but its maximum intensity occurs only over a short pH range, 10·7–11·0. This anion is the most fluorescent form of tryptophan; it has two-thirds the intensity of indole and is about 14 times as fluorescent as aniline. The dianion (XXI) appears at H_0 15. The pK_a values for these changes are given in Table 1.

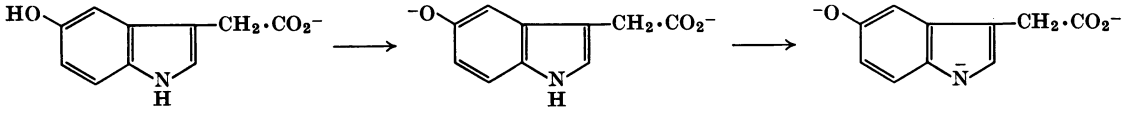
Indol-3-ylacetic acid and 5-hydroxyindol-3-ylacetic acid. Indol-3-ylacetic acid shows the typical indole fluorescence with an indication of the ionization of the carboxyl group (XXIII→XXXIV) in the intensity–pH curve (Fig. 4). The anion (XXIV) is highly fluorescent, 25% more fluorescent than indole. The pK_a values for the above ionizations are given in Table 1. 5-Hydroxyindol-3-ylacetic acid shows, in addition, a fluorescence in acid at 545 mμ (which is discussed below) and a diminution in fluorescence intensity at about pH 10 due to the ionization of the 5-hydroxy group. This fall in intensity occurs at a lower pH than in the other indoles and aniline (Figs. 1, 2 and 3), which suggests that its cause is different, i.e. it is not an excited-state ionization. It can be ascribed to the ionization of the phenolic group, for the pK_a value of 11·2 deduced from this fall in intensity is of the right order for a phenol.

Anisidines. The fluorescence of the methoxyanilines (anisidines) between pH 0 and 14 had been examined earlier in this Laboratory by Rosen & Williams (1961), who showed that *o*- and *m*-anisidine fluoresced maximally at 320 mμ ($\lambda_{exc.}$ 280 mμ) at pH 1 and at 350–355 mμ at pH 7–14, but *p*-anisidine fluoresced maximally at 370 mμ from pH 1 to 14 although the excitation wavelength was 285 mμ at pH 1 and 312 mμ at pH 7–14. Thus *p*-anisidine, but not the *o*- and *m*-isomers, showed excited-state ionization (see Bridges *et al.* 1966) at pH 1. These compounds have now been examined in more detail in view of the possible relation of their fluorescences to that of the hydroxyindoles and in an attempt to explain the acid fluorescence at 520–540 mμ of the 5-hydroxyindoles.

The fluorescence data for the anisidines are shown in Fig. 5. The *o*- and *m*-isomers show a relatively strong fluorescence in acid and their cations







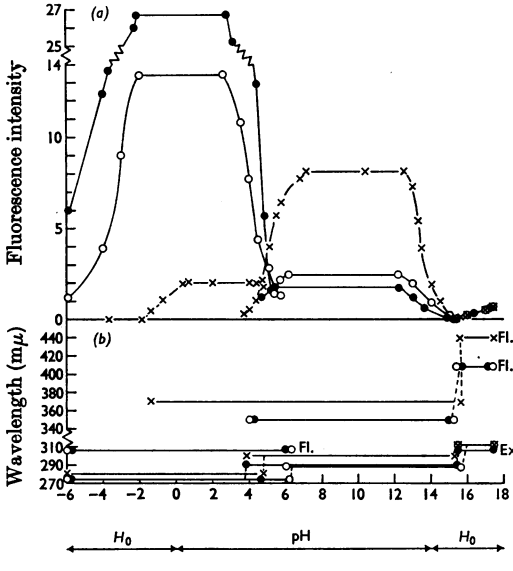
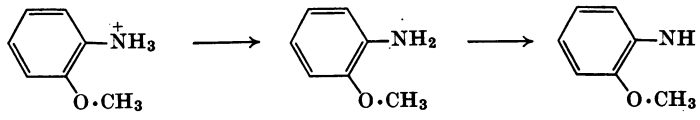


Fig. 5. Variation of (*a*) fluorescence intensity and (*b*) fluorescence (Fl.) and excitation (Exc.) wavelength with pH and H_0 of *o*-anisidine (●), *m*-anisidine (○) and *p*-anisidine (×). Intensity values are arbitrary; that of indole at pH 7 is taken as 100.

(XXXI; *o*-isomer shown) fluoresce maximally from H_0 −2 (9 N-sulphuric acid) to about pH 2·5 at 306 mμ, which is the fluorescence of anisole (see Williams, 1959). They again fluoresce maximally at 350 mμ from pH 6 to 12 in the neutral form (XXXII). The anions (XXXIII) fluoresce weakly at 408 mμ in strong alkali. *p*-Anisidine, however, does not show the anisole fluorescence in acid, but a weak fluorescence at 370 mμ which is that of un-ionized *p*-anisidine. The *p*-isomer thus shows the same fluorescence from H_0 −2 to 15 (in fact a very weak fluorescence persists to H_0 −8·7 or 36·5 N-sulphuric acid), but the excitation wavelength changes at about pH 4 from 280 to 300 mμ. The anion of *p*-anisidine fluoresces maximally at 440 mμ and appears at H_0 15·6. It is noteworthy that the intensity of fluorescence of the neutral form of *p*-anisidine is about four times that of its isomers. The pK_a values of the cations are given in Table 2, the values for the *o*- and *m*-isomers being estimated from the fall in the intensity of the cation fluorescence at 298 mμ and the value for the *p*-isomer from the intensity curve for the appearance of the neutral form at 370 mμ.

Hydroxyindoles and their derivatives. Udenfriend *et al.* (1955) showed that the fluorescence of 5-hydroxytryptamine was different from that of indole in that it showed in 3 N-hydrochloric acid (H_0 −0·6) a visible fluorescence at 550 mμ. This fluorescence is characteristic of 5-hydroxyindoles and is also shown by 5-alkoxyindoles such as 5-methoxyindole, but not by 5-methylindole (see Fig. 2).

Williams (1963, p. 233) reported that, whereas 2-, 4- and 7-hydroxyindoles were non-fluorescent, 3-, 5- and 6-hydroxyindoles were fluorescent in aqueous



(XXXI) (XXXII) (XXXIII)

(*o*-, *m*-, 306 mμ) (*o*-, *m*-, 350 mμ) (*o*-, *m*-, 408 mμ)
(*p*-, non-fluorescent) (*p*-, 370 mμ) (*p*-, 440 mμ)

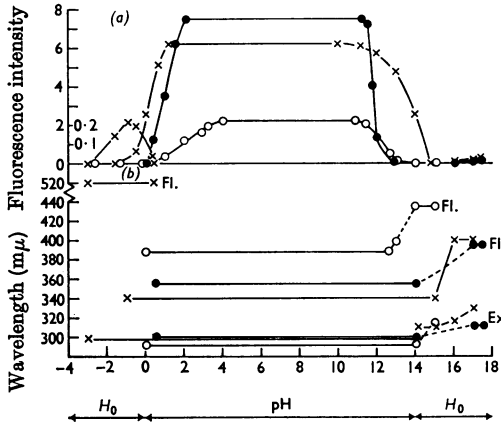
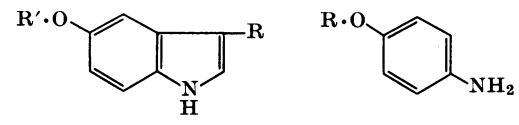


Fig. 6. Variation of (*a*) fluorescence intensity and (*b*) fluorescence (Fl.) and excitation (Exc.) wavelength with pH and H_0 of 6-benzyloxyindole (●), 5-benzyloxyindole (×) and 3-acetoxyindole (○). The fluorescence intensity of 5-benzyloxyindole at 520mμ from H_0 −3 to pH0·5 is shown at ten times the actual values as indicated by the scale on the right side of the intensity ordinate. Intensity values are arbitrary; that of indole at pH7 is taken as 100.

solution. This type of compound has now been examined in more detail. 2-Hydroxyindole occurs in the lactam form, oxindole, which is non-fluorescent in aqueous solution from H_0 −2 to 16 in concentrations up to 100 μg./ml. 4-Benzyloxyindole at 100 μg./ml. is very weakly fluorescent from H_0 −1 to pH13 at 310mμ ($\lambda_{exc.}$ 290mμ) and 4-hydroxydimethyltryptamine (psilocin) is similar. 7-Benzyloxyindole (140 μg./ml.) is similarly weakly fluorescent at 310mμ ($\lambda_{exc.}$ 280mμ). These fluorescences are typical of phenol and anisole.

The fluorescence characteristics of 3-acetoxyindole and 5- and 6-benzyloxyindole are shown in Fig. 6. These characteristics are similar to those of indole insofar as they possess non-fluorescent cations and fluorescent neutral forms and anions. The numerical values deduced from these data are given in Table 2. 5-Benzyloxyindole, however, differs in that it shows a weak fluorescence in acid at 520mμ.



(XXXIV) (XXXV)

The 520–540mμ fluorescence of 5-hydroxyindoles. The following 5-hydroxyindoles were examined and found to show the 520–540mμ fluorescence: 5-hydroxyindole (R=R′=H in XXXIV), 5-methoxyindole (R=H; R′=CH_3), 5-benzyloxyindole (R=H; R′=$C_6H_5\cdot CH_2$), 5-hydroxydimethyltryptamine [R=$[CH_2]_2\cdot N(CH_3)_2$; R′=H; bufotenine], 5-hydroxyindol-3-ylacetic acid (R=$CH_2\cdot CO_2H$; R′=H) and 5-hydroxytryptophan [R=$CH_2\cdot CH(NH_2)\cdot CO_2H$; R′=H]. This fluorescence for these compounds, which appears irrespective of whether the acid is aqueous or ethanolic sulphuric acid or hydrochloric acid, is shown in Fig. 7. Various considerations have suggested that the 520–540mμ fluorescence is an excited-state phenomenon involving the cationic form of the 5-hydroxyindole, and the evidence for this is now considered.

The 5-hydroxyindoles (XXXIV) are comparable with *p*-anisidine (XXXV), 4- and 6-hydroxyindoles with *m*-anisidine and 7-hydroxyindole with *o*-anisidine. Of the anisidines only the *p*-isomer shows excited-state ionization (pK_a^* 0·8) in acid solution. The *o*- and *m*-isomers, however, fluoresce in acid, but as anisole, and it would appear that the effect of the $\overset{+}{N}H_3$ group is slight (cf. the anilinium cation, which is non-fluorescent). Only in *p*-anisidine is there possible a marked interaction between the $\overset{+}{N}H_3$ and O·CH_3 groups through resonance structures such as (XXXVI), which may be important for excited-state ionization. In the quinoline series, 6-hydroxyquinoline corresponds with 5-hydroxyindole, and of the hydroxyquinolines only 6-hydroxyquinoline has been found to fluoresce with a long wavelength, i.e. 550mμ ($\lambda_{exc.}$ 350mμ) (Williams, 1959). This has been confirmed in this Laboratory by J. W. Bridges (unpublished work), who found 6-hydroxyquinoline to fluoresce at 540mμ ($\lambda_{exc.}$ 340mμ) from H_0 −1·1 to pH4·6 and

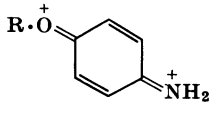
Table 2. *Fluorescence of the anisidines, hydroxyindoles and indolenines*

See the text for concentrations; see Table 1 for other conditions.

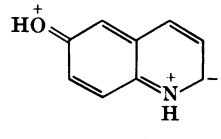
Compound	Molecular species	Wavelength (mμ)			Relative fluorescence intensity		Absolute fluorescence efficiency (%)	pH range of maximum fluorescence	pK_a	
		Of max. absorption	Of max. excitation	Of max. fluorescence	Observed	Corrected			Fluorescence intensity	Albert & Serjeant (1962)
o-Anisidine	Cation	271	274	306	25·4	34·6	17·6	H_0 −2·1–pH 2·8	4·4	4·49
	Neutral form	278	290	350	1·75	1·6	0·70	pH 4·4–12·2		
	Anion	~285	306	408	0·6	0·4	0·20	at H_0 17·4		
m-Anisidine	Cation	267	274	306	13·4	18·3	9·3	H_0 −2·0–pH 2·6	4·2	4·20
	Neutral form	279	288	350	2·45	2·35	1·2	pH 6·2–12·2		
	Anion	287–294	312	408	0·7	0·4	0·2	at H_0 17·4		
p-Anisidine	Cation	269	—	—	—	—	—	—	5·2	5·29
	Cation (excited)	269	280	370	2·0	2·4	1·27	pH 0·7–4·2	−0·8	
	Neutral form	293	300	370	8·1	5·8	3·6	pH 7·2–12·5	15·5	
	Anion	301	312	440	0·7	0·4	0·2	at H_0 17·4		
Oxindole (2-oxindolenine)	Neutral form	280	—	—	—	—	—	—	—	—
4-Benzyloxyindole	Neutral form	~285	290	310	0·02	0·02	0·01	—	—	—
4-Hydroxydimethyltryptamine (psilocin)	Neutral form	~283	292	314	0·07	0·07	0·2	—	—	—
7-Benzyloxyindole	Neutral form	~287	290	310	0·03	0·04	0·02	—	—	
3-Acetoxyindole	Cation	279	—	—	—	—	—	—	2·0	—
	Neutral form	279	292	388	2·2	1·9	1·0	pH 4·0–10·9		
	Anion	~305	315	435	unstable		—			
6-Benzyloxyindole	Cation	291	—	—	—	—	—	—	1·1	
	Neutral form	292	300	355	7·5	5·4	2·5	pH 2·2–11·4		
	Anion	305	312	395	0·2	0·1	0·2			
5-Benzyloxyindole	Cation	~292	—	—	—	—	—	—		
	Cation (excited)	~292	298	520	0·2	0·11	0·2	at H_0 −0·9		
	Neutral form	~293	298	340	6·2	5·2	2·1	pH 1·3–11·3		
	Anion	~295	310–330	400	0·25	~0·12	0·2	at H_0 17·4		
5-Methoxyindole	Cation	~293	—	—	—	—	—	—		
	Cation (excited)	~293	296	520	4·2	3·3	1·0	at H_0 −0·7		
	Neutral form	~292	296	388	125	101	46·0	pH 2·1–10·4		
	Anion	~298	~306	402	35·0	23·0	10·5	at H_0 17·4		
3,3-Dimethyl-5-methoxyindolenine	Cation	242	—	—	—	—	—	—		
	Neutral form	278	305	372	~0·5		0·4			
3,3-Dimethylindolenine	Cation	238	—	—						
	Neutral form	275	303	350	<0·1	—	0·02	—		
Physostigmine (eserine)	Cation		—	—	—	—	—	—		
	Neutral form		265	315	~0·3	—	0·1	—		

who regards resonance contributions from the quinonoid type (XXXVII) as important in this long-wave fluorescence.

The wavelength (520–540mμ) of the acid fluorescence of the 5-hydroxyindoles might suggest that a polymer is involved, since indoles are known to polymerize in acid solution (Albert, 1959). However, this is apparently not the case here, since when the pH is raised the fluorescence disappears and then reappears again on acidification. We suggest that this fluorescence is due to a reaction in the excited state involving the excited 5-hydroxyindole cation. The evidence for this suggestion is based on the findings that: (1) there is no change in the absorption wavelength (270mμ), which is the same in acid as in neutral solution; (2) there is a very large Stokes shift, > 10000 cm.$^{-1}$ (Bridges *et al.* 1966), indicating a large transfer of energy; (3) the process is diffusion-dependent. The last point is supported by the finding that the fluorescence is emitted in ethanolic 3N-sulphuric acid at 20° (room temperature) but not at −196° (liquid nitrogen) when the solution is solid; and when this solvent is replaced by the more viscous glycerol–3N-sulphuric acid at 20° the fluorescence intensity falls to 25% of that in ethanolic 3N-sulphuric acid.



(XXXVI)



(XXXVII)

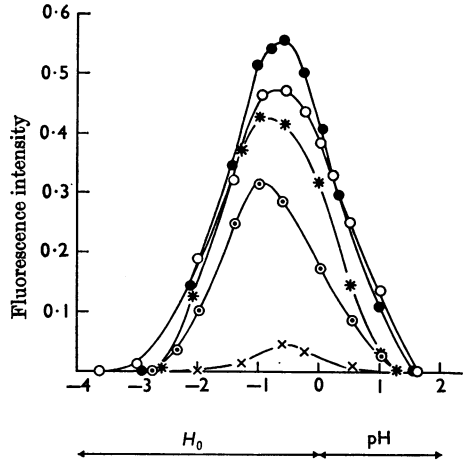
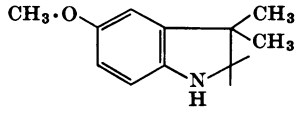


Fig. 7. Variation of the fluorescence intensity of some 5-hydroxyindole derivatives with pH and H_0 over the range H_0 −3·5 to pH 1·6: 5-hydroxyindole (●); 5-methoxyindole (○); 5-hydroxyindol-3-ylacetic acid (*); 5-benzyloxyindole (×); 5-benzyloxytryptamine oxalate (⊙). Intensity values are arbitrary; that of indole at pH 7 is taken as 100.

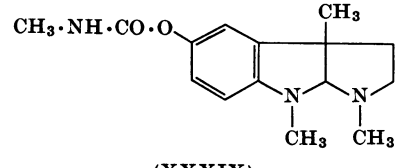
The 520–540mμ fluorescence involves the whole of the 5-hydroxyindole molecule, for if the pyrrole ring is partially reduced it disappears, and it is not shown by 5-methoxy-3,3-dimethylindolenine (XXXVIII) and physostigmine (XXXIX) (see Table 2).

According to Weller (1961) the reactions that can occur in the excited state are: (1) complex-formation, which can include polymerization and solvent–solute interaction; (2) proton transfer; (3) electron transfer. In the present case, polymerization can be eliminated because it has been found that the intensity of the 520mμ fluorescence of 5-methoxyindole reaches a maximum when the concentration reaches 15μg./ml. and then diminishes when the concentration is increased above this value. If polymerization had occurred then the fluorescence intensity would be expected to show a marked increase at higher concentrations, e.g. 100–200μg./ml. (cf. Forster & Kasper, 1955). If solvent–solute interaction were involved then fluorescence wavelengths would depend on the solvent used (Van Duuren, 1963), but this fluorescence had the same wavelength in water, methanol or ethanol made acid with hydrochloric acid or sulphuric acid. If electron transfer (true quenching) had been involved then the production of a new fluorescence would not be expected (Weller, 1961).

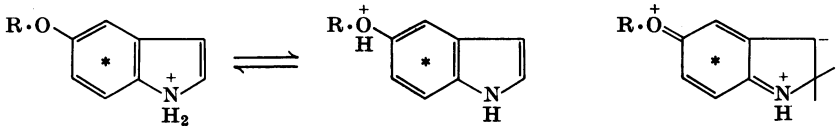
It is therefore suggested that the reaction in the excited state is proton transfer similar to that (protomeric isomerization) suggested by Weller (1961) for salicylic acid. In the excited cation (XL) proton transfer could occur to give the excited oxonium ion (XLI) through an excited inter-



(XXXVIII)



(XXXIX)



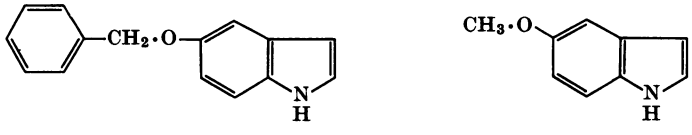
(XL) (XLI) (XLII)

Table 3. *Fluorescence and phosphorescence of benzyloxy and methoxy derivatives of aniline and indole*

The compounds (50 μg./ml.) were dissolved in ethanol.

Compound	Wavelength (mμ) Of max. excitation	Of max. fluorescence	Of max. phosphorescence	Fluorescence intensity* At 20°	At −196°	Phosphorescence intensity (−196°)
p-Methoxyaniline	300	365	440	8·1	10·0	5
p-Benzyloxyaniline	300	365	445	1·0	9·6	5
5-Methoxyindole	280	335	435	135·0	160·0	36
5-Benzyloxyindole	280	330	435	6·2	152·0	35

* Fluorescence intensity of indole (1 μg./ml.) at 20° and pH 7 was taken as 100.



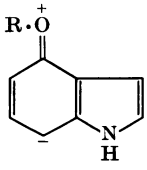
(XLIII) (XLIV)

mediate such as (XLII). Therefore (XLI) is regarded as the species that emits the 520–540 mμ fluorescence. It will be recalled that indole cations such as (XL) do not emit fluorescence. Oxonium ions are well known as derivatives of ethers to exist in acid solution (Hückel, 1955). Proton-transfer processes are often inefficient (Weller, 1955) and therefore a large amount of energy is lost between absorption (by the cation) and emission (by the oxonium ion). The energy emitted as fluorescence is small compared with that absorbed and hence the wavelength of fluorescence is considerably displaced from that of absorption.

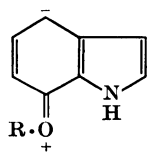
5-Benzyloxy- and 5-methoxy-indole. The intensity of fluorescence of 5-benzyloxyindole (XLIII) is, on an equimolar basis, less than one-tenth of that of 5-methoxyindole (XLIV) (see Table 2), although their extinction coefficients of light-absorption are identical ($3{\cdot}62 \times 10^3$). This may be due to a lack of rigidity in the former molecule as a result of the vibrations ('flopping about') of the large benzyl substituent. These vibrations would tend to use up energy gained by the molecule when it absorbs light and less would be available for emission as fluorescence. The same phenomenon was also observed with *p*-benzyloxyaniline when compared with *p*-methoxyaniline, for these two compounds fluoresce maximally at 365 mμ, but the intensity of the latter is eight times that of the former (Table 3).

If the view that the fluorescence intensity of (XLIII) is due to loss of absorbed energy in this way is accepted, then freezing the solution should prevent these vibrations and the fluorescence intensity of (XLIII) should be nearly the same as that of (XLIV). As Table 3 shows, the fluorescence intensity of (XLIV) at −196° (liquid nitrogen) is higher than at 20°, but that of (XLIII) at −196° is more than 25 times that at 20° and is nearly equal to that of (XLIV). Table 3 also shows that *p*-benzyloxy- and *p*-methoxy-aniline behave similarly. It is possible that the lower intensity of fluorescence of the benzyloxy derivatives at 20° is due to loss of energy in the excited state as phosphorescence (triplet state). If this were so, the benzyloxy compounds should be more phosphorescent than the methoxy compounds. Table 3 shows that this is not so.

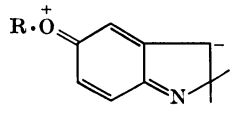
4-, 5-, 6- and 7-Benzyloxyindoles. The 5- and 6-benzyloxyindoles are 100–200 times more intensely fluorescent than 4- and 7-benzyloxyindoles (see Table 2). Further, the 5- and 6-isomers show an indolic fluorescence whereas the 4- and 7-isomers



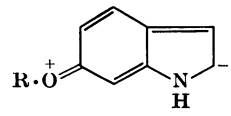
(XLV)



(XLVI)



(XLVII)



(XLVIII)

Table 4. *Stokes shifts for aniline and indole derivatives*

Stokes shift ($cm.^{-1}$) $= 10^7[(1/\lambda_{exc.}) - (1/\lambda_{fl.})]$. —, Form either non-fluorescent or does not exist.

Compound	Cation	Excited cation	Neutral form	Anion
Aniline	—	10100	5400	~10390
o-Anisidine	4220	—	5910	8170
m-Anisidine	4790	—	6150	7540
p-Anisidine	—	10140	7100	10490
Indole	—	—	8870	10710
5-Hydroxyindole	—	15430	5070	8230
5-Methoxyindole	—	15130	4510	10420
5-Benzyloxyindole	—	15020	4840	8900
5-Hydroxyindol-3-ylacetic acid	—	15210	4820	~6940
5-Hydroxydimethyltryptamine	—	15310	5310	8706
4-Benzyloxyindole	—	—	2830	—
6-Benzyloxyindole	—	—	6080	8470
7-Benzyloxyindole	—	—	2830	

show a phenolic fluorescence. One reason for these differences may lie in the types of resonance forms contributing to the fluorescence. The main quinonoid resonance structures contributing to fluorescence for each of these isomers are (XLV) for 4-, (XLVI) for 7-, (XLVII) for 5- and (XLVIII) for 6-isomers. (XLV) and (XLVI) would produce a phenolic fluorescence since only the benzene ring is involved in the quinonoid form, whereas in (XLVII) and (XLVIII) both rings partake in the quinonoid structure.

Stokes shifts. The Stokes shifts (Bridges *et al.* 1966) for a number of relevant compounds are shown in Table 4. The value of the shift is a measure of the energy difference between the ground state (absorption wavelength) and the lowest vibrational level of the first excited state (fluorescence wavelength). For molecules in which no reaction occurs in this excited state, the Stokes shifts are in the region of 2000–5000 $cm.^{-1}$. A shift greater than this can be regarded as indicative of a reaction occurring when the molecule is in the excited state. This reaction robs the excited molecule of some of its absorbed energy and less is available for emission as fluorescence. Emission now occurs from a new species formed during the reaction. The new species may be an ion as the result of excited-state ionization or a product of the excited-state molecule and the solvent, or some other product. Solvent–solute interaction in the excited state has been shown by Van Duuren (1963) to occur with indole in a number of solvents, the interaction being least with cyclohexane and greatest with water. In water indole fluoresces at 350 $m\mu$ ($\lambda_{exc.}$ 285 $m\mu$) and in cyclohexane at 297 $m\mu$ ($\lambda_{exc.}$ 285 $m\mu$).

In Table 4, the excited cations show large Stokes shifts and the possible reactions involved have already been discussed. Among the neutral forms quoted in Table 4, those of *p*-anisidine and indole show large shifts and for indole this appears to be due to solvent–solute interaction. Large shifts are also shown for the anions, but here interpretation is difficult since it cannot be certain that the absorption spectrum measured at H_0 17·4 (10 N-potassium hydroxide) is entirely that of the anion. However, solvent–solute interaction is possible.

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